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Short Communication / Kort Mededeling

Comparison of two methods for estimating the size of the viable seed bank of two plant communities in the Strandveld of the west coast, South Africa

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Due to the important role of the soil seed bank in revegetation studies, the viable seed content of two sites at Brand se Baai (Strandveld of the west coast, South Africa) was estimated by means of the emergence and flotation methods. Most of the seeds were found to be small and only 'apparent viability' was determined after flotation had been completed. The average size of the seed bank differed significantly between the two sites and decreased with increased sampling depth. The flotation method yielded a significantly larger number of seeds per square metre than the emergence method. A combination of these two methods is, therefore, recommended for estimating the size of the germinable seed bank of the Brand se Baai area, due to the problems inherent to each method.

As gevolg van die belangrike rol wat die saadbank in die grond speel in plantegroei-hervestigingstudies, is die lewensvatbare saadinhoud van twee lokaliteite by Brand se Baai (Strandveld van die weskus, Suid-Afrika) deur middel van saailingopkoms en flotering bepaal. Die meeste sade was klein en slegs die 'waarskynlike lewensvatbaarheid' is bepaal nadat flotering voltooi is. Die gemiddelde grootte van die saadbank het betekenisvol verskil tussen die twee lokaliteite en het afgeneem met 'n toename in monsterdiepte. 'n Groter aantal sade per vierkante meter is deur die floteringsmetode opgelewer as deur die saailingopkomsmetode. As gevolg van probleme eie aan die twee metodes word 'n kombinasie van die twee metodes dus vir die bepaling van die grootte van die kiemkragtige saadbank van die Brand se Baai gebied aanbeveel.

Keywords: Emergence, flotation, revegetation, seed bank.

The term 'soil-stored seed bank' has been widely adopted to denote the reserves of viable seeds present in the soil and on its surface (Roberts 1981). 'Seed' is used in the broad sense to describe both true seeds and fruits, but not spores or propagules that are produced vegetatively.

The soil seed bank is composed of: (a) a transient component, made up mostly of seeds at the soil surface that are capable of immediate germination, a few of which remain viable for more than a year, and (b) a persistent component consisting of seeds that may remain viable for several years (Thompson & Grime 1979; Graham & Hutchings 1988).

Extracting seeds from the soil is both time and labour-consuming and the results are influenced by sampling techniques, the time of sampling and methods used to determine seed numbers (Bigwood & Inouye 1988; Benoit *et al.* 1989; Simpson *et al.* 1989; Gross 1990). Estimating the size of the viable seed bank is done either by placing the soil samples under conditions suitable for seed germination (Chippindale &

Milton 1934; Feast & Roberts 1973; Baskin & Baskin 1978; Archibold 1981; Howe & Chancellor 1983; Graham & Hutchings 1988; Granström 1988; Poiani & Johnson 1988; Coffin & Lauenroth 1989; Levassor *et al.* 1990; Matlack & Good 1990), or by using physical methods to separate seeds from the soil particles based on differences in size and/or density (Malone 1967; Price & Reichman 1987; Henderson *et al.* 1988; Benoit *et al.* 1989). Only a few studies have compared the results of different methods to estimate the soil seed bank in a single community (Roberts 1981; Gross 1990; Pierce & Cowling 1991; Brown 1992). Such comparisons are necessary to determine the relative accuracy of different methods for estimating specific characteristics of the soil seed bank (Gross 1990).

Ecologists and evolutionary biologists have become increasingly aware of the role that seed banks can play in maintaining ecological (species) and genetic diversity in populations and communities (Gross 1990). The seed bank of a community represents the 'memory' of previous conditions and plays an important role in determining the future vegetation especially after natural or deliberate perturbation (Roberts 1981; Coffin & Lauenroth 1989).

Along the western coast of South Africa, mining of the rich heavy mineral deposits will destroy all the standing vegetation. To revegetate the area, a layer of topsoil will be replaced in the prospect that the natural vegetation will be regenerated from the soil-stored seed bank.

The aim of this pilot study was to compare two methods, *viz.* emergence and flotation, to estimate the size and depth distribution of the seed bank at two localities at Brand se Baai. Based on these results, recommendations are made here on which method would be best suited for future studies to investigate the seed bank dynamics of all the plant communities of the area. These future studies will aim at developing models that can predict the outcome of the revegetation process in the different plant communities at Brand se Baai.

A preliminary survey revealed that at least two main plant communities will be included in the first area to be mined at Brand se Baai (31°18'S, 17°54'E). These two communities have provisionally been classified as the *Berkheya fruticosa – Osteospermum oppositifolium* Community (site 1) and *Chaetobromus dregeanus – Odyssea paucinervis* Community (site 2) (De Villiers *et al.* 1993). The most prominent difference between these two communities is the abundance of the grass *Odyssea paucinervis*, which is almost absent at site 1 but has an estimated canopy cover of 15 – 20% at site 2.

To estimate the size of the seed bank of the above-mentioned communities, soil samples were taken at one locality in each community in late summer. Sixty soil cores (diameter 56 mm) were taken for each locality and at three different depths: 0 – 50 mm, >50 – 150 mm and >150 – 300 mm. The soil samples were stored dry at ambient temperatures (Feast & Roberts 1973) until the onset of the rainy season at the study area (approximately two months), before the different methods were applied.

For the seedling emergence experiment, a 200-g subsample from each soil sample was spread thinly on top of sterile sand in a 1-dm^3 pot and placed in a phytotron at a day temperature of 20°C and a night temperature varying between 10 and 15°C. These controlled conditions were found to be favourable for the germination of several species from the Strandveld (Grundling 1992; De Villiers 1993). The artificial light, with a daylength of 12 h, had a photosynthetic photon flux density of approximately 43 μmol m^{-2} s^{-1}. Samples were watered daily and emerged seedlings were marked and counted every second day for the first month and weekly for the following 11 months. Half-strength Arnon and Hoagland's complete nutrient solution (Hewitt 1952) was applied fortnightly. After six months, all pots were moved outdoors to attain a change in environmental conditions which may favour the germination of seeds of several species. Watering and counting of emerged seedlings continued as before.

For the flotation experiment, a 100-g subsample from each of the soil samples was suspended in a saturated potassium carbonate solution (Roberts 1981; Janse van Rensburg 1982). Inorganic particles were removed by draining, while the remaining solution, containing the organic matter, was filtered through Whatman No. 1 filter paper. The organic matter was examined under a stereomicroscope and all seeds which seemed to be intact and which resisted gentle pressure were removed and counted. These seeds were regarded as 'apparently viable' *sensu* Roberts (1981). Chemical viability tests were not performed due to the small nature of the seeds, so viability could not be assured. Due to the high frequency of dormancy in the seeds of Namaqualand species, germination tests would not have given satisfactory estimates of the number of viable seeds and were therefore not performed.

Results were analysed statistically using the one-way and multi-factor analysis of variance (ANOVA) and LSD (Least Significant Difference) multiple-range test of the Statgraphics 5.0 computer program, to test for significant differences at $\alpha =$ 0.05 (Statgraphics 5.0, 1989, STSC Inc., USA).

On average, more seedlings emerged at site 1 than at site 2 (Table 1a). In spite of the abundance of *O. paucinervis* at site 2, no seedlings of this species were recorded in these soil samples. Either the abundance of *O. paucinervis* seeds was very low in the soil samples, or the specific conditions, suitable for germination of this species's seeds, had not been met.

A significant decrease in the average number of emerged seedlings with increasing depth was observed. In relatively undisturbed habitats, most seeds are usually found near the soil surface (Bigwood & Inouye 1988; Graham & Hutchings 1988; Kemp 1989) and for most purposes sampling to a depth of no greater than 50 mm should be adequate (Roberts 1981).

The number of seeds extracted by flotation from the soil samples taken at a depth of 0 – 50 mm, was significantly higher at site 1 than at site 2 (Table 1a), although the number of seeds, at depths of >50 – 150 mm and >150 – 300 mm, did not differ significantly between the two sites. As was the case with the emergence method, no viable seeds of *O. paucinervis* were present in the soil samples. The germination requirements of this species were therefore not the limiting factor, but rather the low abundance of its seeds in the soil samples or the transient nature of its seed bank. For both sites, the number of extracted seeds decreased significantly with increasing depth (Table 1a).

These two methods gave highly significant different estimates of the size of the seed bank of two plant communities at Brand se Baai (Table 1b). For a depth of 300 mm, the total number of germinable seeds estimated by the emergence method was 13 512 m^{-2} for site 1 and 4 901 m^{-2} for site 2. The corresponding values for the flotation method were 62 285 m^{-2} for site 1 and 47 262 m^{-2} for site 2. This difference in seed number obtained by emergence and flotation is ascribed to the role of seed dormancy in the emergence method. Jensen (in Roberts 1981) found that seedling emergence gave an estimate equivalent to 38% (75% when the species with the highest seed numbers were excluded) of the viable seeds recovered by separation. In this study, the corresponding estimate was even lower at 17.6%.

The number of germinable seeds obtained by the emergence method at Brand se Baai is lower than in the Namaqualand Broken Veld (Van Rooyen & Grobbelaar 1982), where values of up to 41 000 seeds m^{-2} for a depth of 75 mm were obtained by the emergence method. Kemp (1989) stated that North American hot deserts can achieve seed bank sizes varying from 8 000 to 30 000 seeds m^{-2}.

When emergence methods are used, the size of the seed

Table 1a The average number of seeds/seedlings present in 200-g soil samples from two sites at Brand se Baai ($n = 10$)

	Method							
	Emergence				Flotation			
Depth	Site 1	Site 2	Average	LSD	Site 1	Site 2	Average	LSD
0 – 50 mm	18.8 c[a]	6.1 abc	12.45		83.4 e	52.0 d	67.70	
>50 – 150 mm	3.6 ab	0.9 a	2.25	5.32	11.8 abc	15.6 bc	13.70	12.15
>150 – 300 mm	0.2 a	0.6 a	0.40		5.4 ab	3.2 ab	4.30	
Average	7.53	2.53			33.53	23.60		
LSD	4.34				9.92			

[a] Values followed by the same letter do not differ significantly at $\alpha = 0.05$.

Table 1b Summary of the ANOVA statistics of the main effects and their interactions

Source of variation	D.f.[a]	F-ratio	Significance level	Significance
Main effects				
A: Method	1	75.942	0.0000	**[b]
B: Depth	2	75.730	0.0000	**
C: Site	1	7.645	0.0067	**
Interactions				
AB	2	35.136	0.0000	**
AC	1	0.834	0.3728	NS[c]
BC	2	7.315	0.0010	**
ABC	2	1.861	0.1605	NS

[a] Degrees of freedom.
[b] Highly significant.
[c] Not significant.

bank is likely to be underestimated, because the specific germination requirements of all the species present in the soil are not likely to be met by a single prescribed germination treatment. Some seeds might also be in a dormant state, while other seeds do germinate, but for some reason the seedlings do not emerge. The main advantages of the seedling emergence technique are that the effort required is spread over a period, each seedling represents a viable seed, and seedlings are usually easier to identify than seeds. This method is therefore usually preferred for monitoring long-term experiments and for studying seasonal changes in the seed bank.

On the other hand, separation by physical means would be expected to reveal many more seeds than seedling emergence, and seed numbers may be overestimated unless they are adjusted for viability (Gross 1990). However, for many species it is not possible to distinguish between viable and non-viable seeds in the floated samples (Roberts 1981). Flotation methods have the advantage that the results are not influenced by differences in germination requirements and dormancy, but the accuracy of these methods is variable and species with small or cryptically coloured seeds may be missed. Flotation methods are, however, extremely laborious and impracticable for large-scale studies, especially those involving small-seeded species as in this case.

Seed size is another important factor determining the most suitable method to estimate the size of the seed bank of a specific area. Poiani and Johnson (1988) found that the presence of many large seeds made the seed flotation method unreliable, whereas Gross (1990) found small seeds did the same. Roberts (1981) found the problem of isolating seeds to be more difficult when, as is usual, a range of species was present which differed in seed size. In this study, the majority of seeds were found to be very small, which complicated seed isolation, identification and determination of viability after flotation had been completed.

In his study on the comparison of the seed extraction and seedling emergence methods, Brown (1992) found the difference in species composition determined by the two methods to be more alarming than the threefold difference in density. The ability of a particular method to detect a species was apparently a partial function of seed size. With the emergence method a larger proportion of smaller seed species could be detected, whereas the extraction method had the enhanced ability of detecting tree and shrub species which had larger seeds. Pierce and Cowling (1991) reported that species differences in estimates were a function of seed bank size. Species with larger seed banks gave higher estimates by counting than species with fewer soil-stored seeds, which could easily be overlooked during seed counts.

In future studies, emergence methods are recommended as basis to evaluate the short-term outcome of the revegetation process if the soil-stored seed bank is used. For this purpose, soil samples should be taken at different times of the year as well as in different years. To ensure germination of a maximum number of species in these samples, as many as possible varying environmental conditions should be applied for as long a period as possible. However, for revegetation purposes a knowledge of which species are *not* present in the seed bank is as important as the knowledge of which species are present. As illustrated by the example of *O. paucinervis*, the emergence method cannot distinguish between seeds that are dormant and those that are absent. Dormant seeds can play a major part during the revegetation process, once the environmental conditions required to break the dormancy are met. To relate the number of seedlings found by means of the emergence method to the total number of viable seeds, at least a proportion of soil samples will have to be investigated by both methods. Both methods have limitations – when used in combination, however, the two methods can complement each other.

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